May 10, 1954

Dr. P. D. Skaar
Biological Laboratories
Cold Spring Harbor, L.I., N.Y.

Dear Dave:

I hope everything has gone well with Linda and your second-born.

Thanks very much for the succinct summary of your immunogenetic studies. I will give it closer study at my first opportunity, but so far have been able only to glance at it. I think my earlier belated conclusion still holds—that it would have been better to look for crossable strains among materials that had been serologically schematized: it would have put the burden of interpretation of cross-reactions and the like on the schematists. Anyhow, Aleck Bernstein has found a few apparently fertile isolates in the O-55, O-111, 026 series, and is in the midst of the slow job of manufacturing the auxotrophs needed for a more complete analysis.

Should we resuscitate the question of publishing at least two aspects of your work here? The material on the cattle serums is certainly interesting, especially the ingenious application of the twin analyses. This ought to make a quite satisfactory contribution to the Journal of Immunology, but I suggest that you confer directly with Wilmer on this, unless you would prefer that I mediate. Second, of course, is the production of F- by transfer in motility agar. As you know, I had done a fairly extensive series myself late last fall, fully confirming the application, and the method is now in routine use here. It has only occasionally failed, usually by failure to elicit motility. This question comes up, however: I have a distinct recollection that you had recorded that many of the F- isolates from F+ strains proved to be refractory to reconversion (back to F+). This has not been our experience more recently, but before we go into this rather tedious question, I want to be sure of the refractory experiences you have had. Can you dig these out of your notes? Were any of them saved (as W- stocks or otherwise). One possible source of error might be the occurrence of strains that have become almost incompatible, but are still F+ by the criterion of their conversion of other testers. Tom tells me that the stored stock of W-2301 (your 3.26/4, W-1325 H_6 F-) is now F+. But as I mentioned I would like a more complete dissertation from you before tangling with the details of this. ~~~ F-'s obtained from Hfr have, of course, proved refractory (and these, by the way, tell against my suggestion that the conversion consists merely in establishment of optimal conditions for the survival and expression of spontaneous F- variants in a pyrely passive sense), but I had some (not completely convincing evidence that such F- can be <u>temporarily</u> converted by mixed culture with F+ (in menages a trois). Boris Rotman did one more

extended chemostat run with 58-161, but unlike the experiment with Novick, this gave no conversions, so the matter is still wide open. Anyhow, I think that when some of the minor details are clarified in our own minds, it would be appropriate to submit a brief descriptive account as a Note (ca. 500 words) in the Journal of Bacteriology. I have no objection to your unpublishing the same material in MGB, but am not enthusiastically in favor of it.

Right now, I have been continuing the single cell isolations of Hfr x F- zygotes, and you maybbe interested in this reeent finding. In a setup involving motile Hfr x non-motile F- (another line), pairs of conjoined cells are not difficult to find. So far, of 16 such pairs satisfactorily analysed, 13 have shown recombinants among the immediate progeny after disjunction (which takes 30 - 150 minutes). [The other three could also have represented zygotes, of course, which segregated no detected recombinants]. The recombinants so far have always come from the F- exconjugant. The recombination frequency among these cells taken at random is only about 1% under similar conditions. This looks like quite competent evidence for the process of temporary conjugation, with migration of a gamete nucleus from the Hfr to the F- cell. There has been a suspicious amount of lethality among the exconjugants [Conceivably, but improbably, incompatibility is merely a reflection of such lethality]. But there have been enough cases where both exconjugants have survived, though it will be difficult to exclude the possibility that one of the parental cells was already effectively divided. There is really very little more I can say about this at the instant. The mode of conjunction is not yet certain, but still consistent with the pictures of fixed and stained material I had last year.

Before I forget, let me remind you of the major point of this letter-- details on refractory F-.

Yours,

Joshua

Joshua Lederberg